Chapter 13

Oil Production by Microalgae in Outdoor Mass Culture

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Summary

Microalgae, which have been called the most productive biochemical factories in the world, can potentially produce up to 30 times more oil per unit of growth area than land plants. It is estimated that 150–400 barrels of oil per acre per year could be produced with microalgal oil technology, thereby enhancing U.S. energy security. Initial commercialization of this technology is envisioned for the desert Southwest because this area provides high solar radiation and offers flat land that has few competing uses (hence low land costs). Similarly, there are large saline aquifers in the area which could provide a suitable low cost medium for the growth of many microalgae. Recently, microalgal biomass technology has begun to assume an additional importance because of its potential environmental benefits. Global climatic changes attributed to the release of carbon dioxide from fossil fuel combustion have the potential of producing economic and geopolitical effects with a profound impact on the U.S. energy industry. Excess carbon dioxide derived from processed flue gas of a power plant can potentially be used by microalgal biomass facilities because very large quantities of carbon dioxide are required as a nutrient. Consumption of carbon dioxide by the microalgae with the subsequent production of oil-rich biomass would simultaneously reduce the overall carbon dioxide emissions and provide an alternative source of fuel.

Introduction

When stimulated by environmental stress, many species of aquatic microalgae produce lipids, or oils, that can be processed into diesel oil or gasoline. These algae have growth rates as high as five times those

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of most terrestrial plants, and some species flourish in saline or brackish water unsuitable for human or traditional agricultural use. In addition, microalgae require large quantities of carbon dioxide for growth and lipid production, offering a way of reducing the concentration of atmospheric carbon dioxide.

Studies have shown that economical fuel production will require that microalgae be grown in intensive culture in large outdoor ponds. The preliminary design consists of 22.5 cm deep, raceway-shaped ponds (0.25 acre) with a paddlewheel for circulating the water. Carbon dioxide and other nutrients are injected into the culture to optimize algal growth and lipid production.

In previous work, we have collected and studied more than 3000 strains of microalgae from desert and saline environments [1]. From these, various researchers have identified a number of promising lipid-producing species. These organisms grow over a wide range of salinities, produce significant quantities of oils and achieve growth rates of nearly three doublings per day. Some of these species tolerate temperatures of 38 °C or higher.

Research is now focused on applying genetic techniques to enhance the lipid production of microalgae. This effort builds on extensive strain characterization research, as well as biochemical studies of the metabolic pathways for lipid synthesis. Using non-genetic methods, scientists have already improved the lipid content of the cell from the 5–20% found in nature to more than 60% in the laboratory, and more than 40% in outdoor culture.

Microalgae production facilities are well suited to areas with high solar radiation, inexpensive, flat land, adequate saline water, and an inexpensive source of carbon dioxide. The desert Southwest meets all of these requirements. Several large coal-burning power plants in the region offer an excellent potential source of carbon dioxide for large microalgae farms.

Coupling a microalgae farm with a power plant or other source of carbon dioxide provides a way to produce liquid transportation fuels and improve the environment at the same time. The microalgae essentially recycle the carbon dioxide from the power plant's stack gases into a secondary energy product (diesel fuel or gasoline). Although this carbon dioxide is eventually released when the fuel is burned, the process effectively doubles the amount of energy generated for a given quantity of carbon dioxide. Studies have shown that land and saline water are available in New Mexico and Arizona to support extensive microalgae facilities. The carbon dioxide emissions from all the power plants in these two states could be trapped by microalgae farms covering only about

0.25% of the total land area. If this technology is expanded to other states, or projected future capacity is brought on line in Arizona and New Mexico, the farms could supply at least two quadrillion Btu of energy (equivalent to 15% of the gasoline used in the United States) in the form of liquid fuels.

Project Overview

Researchers in the Aquatic Species Project at the National Renewable Energy Laboratory (NREL) focus on the use of microalgae as a feedstock for producing renewable, high-energy liquid fuels such as diesel. It is important for the United States to develop alternative renewable oil sources because 42% of the current energy market in the U.S. is for liquid fuels, and 38% of these fuels are imported. The latter figure is expected to rise to more than 50% soon, thereby increasing the trade deficit and our vulnerability to disruptions in petroleum supplies. In 1979, the U.S. Department of Energy (DOE) and NREL initated the Aquatic Species Project as part of the overall effort in biofuels. The project began to focus exclusively on fuels from microalgae in 1982. Estimates show that the technology being developed by the project can provide as much as seven percent of the total current energy demand.

The project's basic premise is that microalgae, which have been called the most productive biochemical factories in the world, can produce up to 30 times more oil per unit of growth area than land plants. It is estimated that 150–400 barrels of oil per acre per year could be produced with microalgal oil technology. Initial commercialization of this technology is envisioned for the desert Southwest because this area provides high solar radiation and offers flat land that has few competing uses (hence low land costs). Similarly, there are large saline aquifers with few competing uses in the region. These could provide a suitable, low-cost medium for the growth of many microalgae.

Recently, the project has begun to assume additional importance for its potential contribution to the environment. A potential global climate change is projected as a result of the release of carbon dioxide from fossil fuel combustion. Global climate change has the potential of producing economic and geopolitical changes with profound impacts on our economy and the energy industry. The production of diesel fuel by microalgae requires the uptake of very large quantities of carbon dioxide as a nutrient. In areas where microalgae fuel plants operate, and in regions where these plants operate in tandem with fossil fuel plants to scrub carbon dioxide from flue gases, contributions to the greenhouse effect could be significantly reduced.

The project has supported research at NREL, as well as in industry, other government laboratories, and universities.

Project Objectives

Specific long-term objectives of the project are to:

1. genetically engineer microalgae for high lipid production at high growth rates;
2. identify "trigger" points in biochemical pathways of algae that turn lipid production on and off;
3. develop inexpensive, large-scale, outdoor mass culture technologies to grow microalgae;
4. evaluate resource requirements for the growth and environmental impact of microalgae in the desert Southwest of the United States;
5. develop technologies for converting microalgal lipids into high-value liquid transportation fuels, particularly diesel; and
6. transfer the technologies to the private sector for continued development and rapid commercialization by involving industry in the research process as early as possible.

Description of the Aquatic Species Project Elements

Production

Improvements are needed in the algal growth and lipid productivity in order to produce economical liquid fuels from microalgae. The production goal is to reach target growth rates of 50 $g \cdot m^{-2} \cdot d^{-1}$ in outdoor cultures and produce algae cells that contain 50–60% lipid. These algae have been screened to select species that are temperature and salinity tolerant, have high productivities and are good lipid producers. A collection of organisms is being maintained to provide a gene pool for direct exploitation as energy crop organisms in the laboratory and test facility, and also as starting material for genetic engineering. It is also important to understand lipid biochemical pathways in algal cells in order to maximize lipid production and to develop genetic engineering techniques for improving microalgal production and lipid content.

Microalgal strains exist in the NREL culture collection which produce large amounts of lipid, have a high degree of salinity or temperature tolerance, or grow rapidly. Genetic improvements will be necessary to develop strains with the characteristics necessary to meet the overall project technical and economic goals. The genetic engineering process requires methods for getting a gene (DNA) out of the cells (cloning),

modifying the gene in some way and reintroducing it into the microalgae. The modified genes would then confer one of the desired characteristics, such as increased lipid content.

Extraction and Conversion

Methods need to be developed for economical extraction of lipids from microalgae and conversion of lipids to gasoline and diesel substitutes. Untreated lipids have too high an oxygen content and viscosity to be used in standard engines. The primary goal of this effort is to economically convert a high proportion of the algal lipids to diesel fuels and to improve the overall economics by converting the balance of the biomass to biogas or other high energy products.

Engineering Design

The technology to produce economical liquid fuels from microalgae will require the growth of microalgae on a large scale. Systems to maintain optimal levels of nutrients, carbon dioxide and salinity must be developed and tested. The engineering design goal is to develop large-scale outdoor facilities that allow the production goals to be met and to reduce the economic costs of such a system to those targeted by the project's economic analysis. A recent review of progress in this area has been reported by Weissman and Tillet [2].

Analysis

Economic and resource analyses provide input to project management so research directions and priorities can be set. The analysis component's goal is to support the technology development by determining cost goals, economic sensitivities, resource assessments and environmental impacts as new data are developed. To do this, researchers will conduct ongoing economic analyses. Resource and environmental assessments will be conducted to identify potential constraints, identify and address data gaps, and provide project guidance. A major study was completed several years ago by Neenan *et al.* [3].

Recent Project Highlights

Project efforts have been focused most recently on genetic improvement methodology and the development of carbon dioxide sources for microalgal mass culture from flue gas.

Genetic Transformation of Microalgae

Recombinant DNA technologies have been applied successfully in prokaryotes, fungi and higher plants. However, little work has been done with the microalgae, which are potentially capable of producing a wide range of products and have broad ecological importance. These reasons alone are sufficient for studying their molecular biology. The specific interest in this project is in increasing accumulation of lipids by microalgae to achieve higher lipid contents in outdoor mass culture. Our long range objective is to produce alternative fuels from these lipids. The tools of modern molecular biology are being applied to this work in order to isolate some of the key genes that regulate lipid biosynthesis and to find ways to introduce genetic material into the microalgae and have these genes expressed. These methods, once developed, will enable the further development of microalgal biotechnology as a whole.

The key components that require development are methods to introduce foreign genes into microalgae, and the identification and cloning of key genes in photosynthate partitioning (the flow of carbon into lipid versus other cellular components). Transformation of microalgae requires overcoming at least three general problems: 1) introducing DNA into the cells; 2) monitoring gene entry and expression; and 3) stabilizing the foreign DNA in the host cell. In order to have exogenous DNA expressed in microalgae, it is necessary to find ways of introducing recombinant molecules into the cell. A key issue here is finding some way to penetrate the cell covering or cell wall by protoplast production, electroporation or other methods. Several of these approaches for the introduction of genetic material into industrially important microalgae are being pursued. The next step in the transformation process is the short term expression of a gene introduced into the cell. We have had success with the luciferase gene from the firefly. This has proven to be an effective marker gene for transformation in microalgae.

As for higher plants, the cell wall of microalgae presents a significant barrier to the introduction of exogenous DNA into the cells. Plant cell walls can be removed enzymatically to form protoplasts that can then be induced to take up foreign DNA in the presence of polyethylene glycol (PEG) and/or calcium [4]. We have successfully adapted plant protoplast transformation protocols for introduction of a foreign gene (the luciferase gene from the firefly) into the green alga *Chlorella ellipsoidea* [5].

The firefly luciferase transient assay has been used to monitor DNA uptake and expression in a variety of cell types [6,7], including plants [8]. In the presence of ATP, O_2 and Mg^{2+}, luciferase catalyzes the oxidation of luciferin with the concurrent release of a photon of light. Luciferase can thus be detected in crude extracts from cells expressing this gene

by monitoring light production in a scintillation counter or luminometer. Protoplasts were produced from the green alga *C. ellipsoidea* using the procedure of Gobel and Aach [9]. Cells grown to early stationary phase were digested with Cellulysin (Calbiochem, San Diego, CA), a crude preparation of cellulase. Protoplasts, defined as cells that could be disrupted when suspended in water and sonicated, were treated with a plasmid containing the luciferase gene under the control of the CaMV 35S promoter (pDO432; generously provided by David Ow). Following PEG treatment and overnight incubation, luciferase activity could be detected in extracts of the protoplasts. Expression of luciferase was only detectable in cells treated with Cellulysin (protoplasts), and the addition of both carrier DNA and PEG was essential. A time course of expression showed that luciferase was made rapidly (within about seven hours after addition of DNA), but that the activity disappeared over the course of a few days.

These results show that we have accomplished the first steps in the development of a transformation system for *C. ellipsoidea*. Viable protoplasts can be produced in these cells by digesting the cells with Cellulysin. The *C. ellipsoidea* protoplasts can be induced to take up exogenously added DNA and to express a heterologous (*i.e.*, a non-algal) gene. This result is significant as homologous genes were required to achieve transformation in another green alga, *Chlamydomonas reinhardtii* [10], and doubts have been expressed as to whether algae could recognize heterologous genes. Further experiments will be directed toward increasing the level of expression, developing a sensitive selectable genetic marker, stabilizing the foreign DNA within the cell, and promoting recovery and proliferation of stable transformants.

Carbon Dioxide Sources for Microalgal Mass Culture from Flue Gas

Preliminary analysis of the effects of inputs of flue gas components on mass culture was completed, including a consideration of carbon dioxide, heat, nitrogen, acidity and salinity. Waste heat from a power plant would contribute only 3–12% of the total heat input to the pond; the remainder is solar. Nitrogen, water and salinity inputs to the pond are even less important. If SO_x is not neutralized, significant acid may be added. However, sulfur scrubbing is being installed in many plants in order to satisfy clean air requirements.

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